REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments the following commentary.

I. Amendments and Status of the Claims

Claims 1-38 are requested to be canceled without prejudice or disclaimer.

Claims 39-66 are requested to be added. Claim 39 is the only independent claim. It derives from claim 1, claim 5, claim 7, claim 10 and claim 12, but it is directed to RNA. The aforementioned claims support claim 39, except that an additional detection step e) is included. A detection step is supported, for example, by the specification at page 7, line 8. Certain clarifying and functional language is included in claim 39. In step a), support for "freed" is found in the specification at page 7, line 20; chaotropic salt is supported at page 7, lines 24-26; the functioning of the chaotropic salt to denature or degrade proteins, including nucleases is supported at page 7, lines 23-24; restricting RNA loss to 25% is supported by page 15, lines 4-6 and page 8, lines 19-30; and naming the product as a disrupted product sample is supported at page 23, line 13 and generally by the specification's many references to the chaotropic salt as a "disruption reagent," such as at page 7, line 23. In step b), the clarifying term "without washing" is supported by claim 1's wording "without removal of or isolation of said RNA ... molecules from each other or from the other components;" and dilution to 0.05M before addition of reverse transcriptase is supported by the specification at page 9, lines 9-11 and the Examples. Thus, claim 39 does not include new matter.

The claims dependent from claim 39 also do not contain new matter. Claims 40 derives from claim 3, for example. Claim 41 derives from claims 3 and 19. Claim 42 derives from claim 13. Claim 43 specifies that the chaotropic salt concentration prior to heating is at least 2M, a preferred embodiment specified at page 15, lines 1-2. Claim 44 derives from claim 14. Claim 45 derives from claim 3, and the functional language is found at page 8, lines 2-5. Claim 46 derives generally from claim 17, and the act of dissolving in the sample is supported by page 16, lines 9-12. Claim 47 derives from claim 11. Claim 48 derives from claim 2. Claim 49 derives generally from claim 17, and the act of dissolving in the sample is

supported by page 16, lines 9-12. Claim 50 is supported by page 16, line 11. Claim 51 derives from claim 20.

Claim 52 derives from claim 21. Claim 53 is supported by page 15, lines 1-2. Claim 54 and claim 55 derive from claim 13. Claim 56 is supported by page 10, lines 18-20. Claim 57 is supported by page 10, lines 20-24. Claim 58 is supported by page 14, lines 13-14. Claim 59 is supported by page 15, lines 1-2. Claim 60 derives from claim 14. Claim 61 derives from claim 3, and the functional language is found at page 8, lines 2-5.

Claim 62 is supported by page 8, line 4. Claim 63 derives from claim 3. Claims 64 derives from claim 22. Claim 65 is supported by Example 3 at page 23, line 13 to page 24, line 7. Claim 66 is supported by page 17, lines 29-30.

Upon entry of the amendment, claims 39-66 will be pending and subject to examination on the merits.

II. Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 12-23, 37 and 38 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for reciting "at least about."

While not acquiescing in the propriety of the rejection, the new claims do not recite "at least about." Thus, the amendment renders this ground of rejection moot.

III. Claim Rejections – 35 U.S.C. § 102(b)

A. Vodkin *et al.*, BIO TECHNIQUES 17(1): 114-116 (1994)

Claims 1-9, 12, 13 and 37 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Vodkin *et al.*, BIO TECHNIQUES 17(1): 114-116 (1994) as evidenced by U.S. Patent No. 6,762,160 to Barbeau *et al.* Applicants respectfully traverse this ground of rejection.

Vodkin relates to treating mosquitoes in preparation for an RT-PCR assay. *See* Vodkin at pg. 114, center column. Specifically, mosquitoes are triturated, that is ground up/homogenized, in an aqueous solution containing certain reagents (none of which is a

chaotropic agent), which would lyse cells. A reagent containing Tris-HCl, EDTA, and 1% SDS was added to an aliquot of this triturated solution. The mixture was then heated at 94°C for 5 min. An aliquot of this heated solution was diluted 1:10 or 1:100. Finally, the diluted aliquot was reverse transcribed in 10 microliters of RT mix. SDS at 1% is about .03M, as the Examiner correctly calculated.

The Examiner states that SDS is a chaotropic agent, "because it solubilizes membranes, and thereby, disrupts hydrophobic interactions." Office Action at ¶ 4.

New claim 39 requires incubation with a chaotropic salt "having a concentration of at least 2M that denatures or degrades all proteins, including nucleases." Also, the chaortopic salt acts "with no more than twenty-five percent loss of RNA." Vodkin does not teach or suggest such a process.

SDS is not a salt that degrades all proteins, including nucleases. Unlike a true chaotropic agent that unfolds proteins, SDS is an ionic detergent that coats proteins and makes them susceptible to unfolding by heat. Unlike a true chaotropic agent that unfolds proteins, SDS is an ionic detergent that coats proteins and makes them susceptible to unfolding by heat. For instance, it is known from the method of preparing samples for electrophoresis in an SDS denaturing gel that a reducing agent (such as mercaptoethanol) is added to the SDS sample buffer prior to boiling for several minutes. Prior to heating to temperature, RNA present in lysed biological samples would be susceptible to nucleases, if SDS were used in place of a guanidinium salt, and one might lose more than 25% of the RNA. Also, SDS could not be added at 2M concentration (about 70 times as much as Vodkin, or a 70% solution). Indeed, it is highly doubtful that one could make a 2M solution of SDS. In any case, it would not be possible using Vodkin's dilution protocol to reduce the concentration of the detergent to a sufficiently low concentration to prevent inactivation of reverse transcriptase.

Regarding claim 3, the Examiner cites "water" as being a water-miscible solvent. This is untenable on its face. One of skill in the art would clear understand a "water-miscible solvent" to be something other than water.

Regarding claim 4, the Examiner cites Vodkin as teaching serial dilution – first water and then RT buffer. Note that the first dilution, the only dilution prior to addition of reverse transcriptase, is either 10 or 100. If one starts with 2M chaotropic agent or SDS, dilution by 100 yields .02M chaotropic agent or SDS. A number of the new claims require dilution to less than .01M chaotropic agent before addition of reverse transcriptase.

Regarding claim 13, the Examiner concludes that Vodkin's heating for 5 min at 95°C is concentrating. However, Vodkin does not necessarily teach concentrating despite the hearing step. The sample could be covered, which would prevent any of the liquid from being evaporated.

Regarding claim 37, the Examiner states that incubating with the disruption reagent and dilution occur in the same container. New claim 39 requires that everything occur in a single container, and the Examiner states on page 5 that Vodkin utilizes just an aliquot for the RT-PCR, which is of necessity not the same container.

B. Thornhill *et al.*, PRENATAL DIAGNOSIS 21:490-497 (2001)

Claims 1-6, 8-13, and 37 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Thornhill *et al.*, PRENATAL DIAGNOSIS 21:490-497 (2001) in view of U.S. Patent No. 5,407,810 to Builder *et al.* and Barbeau. Applicants respectfully traverse this ground of rejection.

The Examiner argues that Thornhill discloses two alternative methods, one of which employs SDS and the other potassium hydroxide (KOH), that anticipate the claims. The Examiner considers SDS and KOH "chaotropic salts," as recited by the present claims. However, neither SDS nor KOH are "chaotropic salts."

1. KOH Is Not A "Chaotropic Salt"

The claims are now directed to "combining said biological sample with a disruption reagent containing a chaotropic salt ... to produce a disrupted sample containing RNA freed from bound proteins and inactivating nucleases ***with no more than twenty-five percent loss***

of RNA." KOH is not such a "chaotropic salt," because KOH is known to destroy RNA due to its high pH. This is stated in the pending application at page 13, lines 8-9.

2. SDS

Thornhill teaches preparation of the DNA in a sample for PCR, not the RNA in a sample for RT-PCR. Thornhill's first incubation used 5 microliters of a "working solution" of lysis buffer containing 125 micrograms/ml proteinase K and 17 micromolar [.017M] SDS. A washed cell in a 1 microliter microdrop of PBS/PVP was added to 5 microliters of the lysis buffer. The incubation was 1 hr at 37°C followed by boiling for 15 min at 98°C "to prevent further enzyme activity." Nested PCR was then performed. For the first PCR, 20 microliters of PCR reaction mix was added to the tube containing a lysed cell, followed by brief centrifugation and thermal cycling. For the second PCR, a 1 microliter aliquot of the primary PCR reaction product was transferred to another tube.

The Examiner says that SDS is a chaotropic agent and that the lysate is diluted by the PCR mix. The Examiner calculates that dilution of 6 microliters of lysate containing 17 micromolar SDS[1] with 20 microliters of PCR mix reduces the concentration of SDS to .004 M.

Comparing Thorhill's starting concentration of SDS, 0.017 M, with Vodkin's starting concentration of SDS, 0.03 M (1%), shows that the remarks on Vodkin also apply to Thornhill. In short, SDS could not be added at 2 M concentration, as required by the present claims.

In addition, Thornhill employs proteinase K, which degrades proteins. This is a well-nown enzyme, not a chaotropic salt. The presence of both SDS and proteinase K in the lysing reagent would possible destroy more than 25% of the RNA. This is not a problem for Thornhill, because Thornhill is directed to a DNA amplification method. However, the destruction of RNA would be a problem for an RNA amplification method, as claimed.

Regarding claim 3, the Examiner cites "water" as being a water-miscible solvent. This is untenable on its face. One of skill in the art would clear understand a "water-miscible solvent" to be something other than water.

Regarding claim 4, serial dilution, the Examiner cites addition of neutralization buffer, which is part of the KOH embodiment, and therefore not part of the SDS embodiment.

Regarding claim 12, the Examiner takes the position that .017M SDS is "at least about 2M" only because "at least about" is indefinite. The new claims do not recite "at least about." It cannot be said that .017 M is "at least 2M" as new claim 39 requires.

Regarding claim 13, the Examiner states that Thornhill heat, so they therefore concentrate the SDS. This is incorrect. Thornhill heat in a DNA Engine thermal cycler "with heated lid." Thus, the tube is covered rather than open to the atmosphere. Heated lids are used specifically to prevent evaporation and change of concentration of PCR reaction mixtures during heating.

IV. Claim Rejections – 35 U.S.C. § 103

A. Thornhill as evidenced by Builder, Barbeau, and U.S. Patent No. 5,939,259 to Harvey *et al.*

Claims 14-19 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Thornhill as evidenced by Builder and Barbeau in view of U.S. Patent No. 5,939,259 to Harvey *et al.* Applicants respectfully traverse this ground of rejection.

Thornhill in view of Harvey fails to render the claimed invention obvious for at least two reasons. First, Thornhill fails to teach or suggest the claim 39, as discussed above in Section III. Nothing in the secondary references remedies this deficiency. Second, Thornhill would not have led one of skill in the art to prepare a mixture that "is at least semi-dry," as discussed below.

Harvey discloses adding a biological sample to an absorbent material, such a cellulosic paper, impregnated with a chaotropic salt, such as a guanidinium salt. For example, 0.5M to 2M (more broadly 0.1M to 6.0M) guanidine isothiocyanate can be added to paper, which is then dried. "Any nucleic acids present in the biological source can be either eluted or re-solubilized off the absorbent material." Harvey at col. 3, lines 6-10. "Other reagents can be add to the

[1] The concentration of SDS in the reaction is 5/6 of 17 micromolar.

present invention in order to enhance lysis...[f]or example, ... surfactants . . . ." Harvey at col.5, lines 1-6). Methods are disclosed in the Examples. In Example 2, a piece of impregnated paper with the sample dried in it was transferred to a tube containing 100 μl water and heated at 95°C for 30 min. Ten microliters of supernatant was then transferred to a PCR mix for DNA amplification. In Example 3, a similar procedure was used, except that there was a preliminary wash of the sample-containing paper by centrifuging in 0.5 ml (500 μl) water before transferring the paper to the tube containing 100 μl water. In Example 4, the washed/centrifuged paper was transferred directly to a PCR mix without being heated in 100 μl water. Harvey does not disclose processing in a single container, as new independent claim 39 requires.

In addition, Harvey does not teach that when a chaotropic salt is used at high concentration, for example 2M as required by new claim 39, one can use a single container and dilution to process RNA in samples. To the contrary, Harvey teahces washing and transfering to another container. Thornhill, on the other hand, discloses much lower concentration of SDS – and even of KOH (0.2M) – for single-tube processing. Because of these differences, one of skill in the art would have not reason to combine Thornhill and Harvey to arrive at the claimed invention. If combined, however, Harvey teaches that if a high concentration of chaotropic salt is used, there should be a wash step and transfer to a second container. Thus, whether or not Harvey teaches storage of a dried, disrupted sample, one would not have been motivated to use Harvey's initial steps in Thornhill's method.

Regarding claim 18, the Examiner states that Harvey discloses the use of a water-miscible solvent, citing Example 1. Reference to that example shows that the Examiner is again asserting that water is a "water-miscible solvent." As stated earlier, this is not tenable.

B. Thornhill as evidenced by Builder and Barbeau in view of U.S. Patent No. 6,440,725 to Pourahmadi *et al.*

Claims 17-23 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Thornhill as evidenced by Builder and Barbeau in view of U.S. Patent No. 6,440,725 to Pourahmadi *et al.* Applicants respectfully traverse this ground of rejection.

Thornhill in view of Pourahmadi fails to render the claimed invention obvious for at least two reasons. First, Thornhill fails to teach or suggest the claim 39, as discussed above in Section III. Nothing in the secondary reference remedies this deficiency. Second, Thornhill or Pourahmadi would not have led one of skill in the art to prepare a mixture that "is at least semi-dry" using a dry disruption agent, as discussed below.

1. Pourahmadi

Pourahmadi generally relates to a microfluidic device for processing a biological sample through a one-step PCR, which is typically a DNA amplification. Two embodiments are described. The first, shown in Fig. 2, includes a lysing reagent chamber 109, a wash reagent chamber 125, and an elution reagent chamber 127, as well as a lysing chamber 119 containing a cell-trapping filter and a nucleic acid-capture chamber 122 that is connected alternately to a waste chamber 139 or to a PCR reagent chamber 141. Operation, described beginning at the bottom of column 8. Specifically, sample and lysing reagent, chaotropic salt, are mixed and flowed to the lysing chamber, where cells are retained by the filter and lysed. The nucleic acids continue with the lysing reagent to the capture chamber, where nucleic acids are captured, and the lysing reagent continues to the waste chamber. Next, wash reagent is passed through the nucleic acid capture chamber and on to waste in order to remove chaotropic salt. Finally, elution reagent is passed through the nucleic acid capture chamber to pick up nucleic acids and move them to the PCR reagent chamber.

The second embodiment is shown in Figs. 16-18 and described beginning in the middle of column 32. It contains a lysing chamber 173 that contains lysing reagent-impregnated filter paper, a wash reagent chamber 195, two waste chambers 201, 203, and a PCR reagent chamber 179. In operation, a sample is placed onto the filter paper and dried, during which time the cells are lysed and biological molecules are bound to the paper. Next, wash solution is passed through the lysing chamber to a waste chamber to wash out and/or elute processing chemicals. When that waste chamber becomes full, two things happen. First, a heater is activated to release nucleic acid molecules from the filter paper. Second, the flowing wash solution exiting the lysing chamber is redirected to a chip that binds target analyte and thence on to the second waste chamber.

When that waste chamber becomes full, two things happen. First, a heater is activated to release target analyte. Second, the flowing wash solution exiting the chip is redirected to the PCR reagent chamber.

2. Pourahmadi fails to remedy Thornhill's deficiencies

Like Harvey, Pourahmadi teaches that when a high concentration of chaotropic salt is used, as in the second embodiment, washing should be employed to remove the chaotropic salt. For the reasons stated above with respect to Harvey, one of skill in the art would not combine Pourahmadi with Thornhill to arrive at the claimed invention. If combined, the combination teaches away from the invention of independent claim 39, because they suggest, at best, low concentrations of chaotropic agents.

Regarding claims 17-21, the Examiner states that Pourahmadi teaches a dried disruption reagent coated on a surface, citing col. 12, lines 34-50 and col. 16, lines 22-39. Reference to those passages reveals that chaotropic solutions are described as liquid reagents at col. 12, lines 18-20. Enzymes (e.g., proteinase K) are described as dried reagents, but chaotropic salts are not. When, at col. 12, lines 40-41, Pourahmadi states that dried reagents in membranes, such as cellulose paper, can effect lysing, that must be a reference to proteinase K, which col. 16, lines 31-34 states is "often used." Thus, Pourahmadi fails to teach or suggest the use of dried chaotropic salt. Nor is the reference seen to disclose even proteinase K as a coating on a surface of a container. Instead, Pourahmadi discloses proteinase K as impregnated in filter paper disposed within the container.

V. New Claims

Claim 39 is now the sole independent claim. It recites "a single-container method," use of a "chaotropic salt" that "denatures or degrades proteins," at a concentration of "at least 2M," dilution "without first separating the RNA from . . . said chaotropic salt," and freeing and reverse transcribing RNA.

Vodkin does not teach or suggest claim 39. Vodkin fails to disclose use of a "chaotropic salt" at 2 M that denatures or degrades proteins. Rather, as indicated above, SDS is a detergent

that coats proteins and renders the susceptible to heat degradation. Further, SDS is used at a low concentration of 0.03M, and its activity would not be eliminated were it used at 2M, assuming *arguendo* that such a concentration could be accomplished. Also, Vodkin does not disclose processing in a single container. Use of an aliquot is specifically recited.

Thornhill does not anticipate claim 39. It does not disclose use of a "chaotropic salt" at 2M that denatures or degrades proteins. It discloses use of KOH, which is not suitable for RNA and is not a chaotropic salt, at 0.2M concentration, or proteinase K with SDS at low concentration, about .017M.

Harvey and Pourahmadi, either alone or in combination with Thornhill, do not anticipate claim 39 for at least the reason that both references disclose methods that include washing. Also, Harvey does not disclose a single-container method.

The cited references also do not render claim 39 *prima facie* obvious. Neither Vodkin nor Thornhill teach or suggest the use of a 2M chaotropic salt that denatures or degraded proteins, and only Thornhill discloses a single-container method. Both Harvey and Pourahmadi teach that when using high concentration a chaotropic salt that denatures or degrades proteins, the process includes washing. In fact, Pourahmadi specifically teach that the purpose of washing is to remove chaotropic salt. The references in combination do not suggest that one could substitute for a low concentration of SDS (plus heat) a high concentration of a chaotropic salt that denatures or degrades proteins without washing to remove the chaotropic salt. A person of ordinary skill in the art would not have been guided by these references to the process of new claim 39.

CONCLUSION

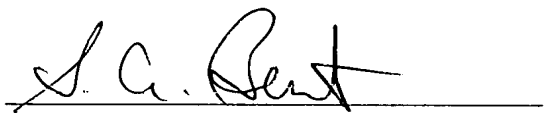
Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date November 1, 2007

By 

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